An *Armillaria* survey in Mexico: A basis for determining evolutionary relationships, assessing potentially invasive pathogens, evaluating future impacts of climate change, and developing international collaborations in forest pathology

Phil Cannon, Ned B. Klopfenstein, Mee-Sook Kim, John W. Hanna, Dionicio Alvarado Rosales*

Abstract--In September 2007, a collaborative effort was made to survey *Armillaria* species in three general areas of south-central Mexico. Collected *Armillaria* isolates will be subjected to DNA analyses to examine genetic relationships with other *Armillaria* species. These studies will provide baseline information for examining evolution of *Armillaria* spp., assessing potential for hybridization or invasive species risk of *Armillaria* spp., and evaluating potential impacts of climate change. During this trip, observations were also made that may provide insights to other forest diseases, such as Annosus root disease, white pine blister rust, dwarf mistletoe, oak wilt, and scorch. Because of common research interests in forest pathology, it seems essential that collaborations are strengthened between the USA and Mexico.

Overview of the Trip

During 2007 September 9–26, a trip was taken to Mexico in search of *Armillaria* spp. in forest areas south and east of Mexico City. The locations visited during this trip are shown in figure 1 and listed in table 2 along with their respective elevations. Generally, collection sites were located between 1,300 and 3,600 m (4,300 to 11,800 ft) above sea level. As a general rule, the closer the location to the Gulf of Mexico, the higher the annual rainfall. With respect to the effect of elevation, higher elevations typically are associated with less rainfall (the moisture gets dropped at lower elevations), and rain is limited at elevations above ca. 3,200 m. As expected, forest areas on the Pacific side also receive less rainfall due to a pronounced rain shadow effect.

Because the 12 different locations varied in elevation, precipitation, and soils, they also varied in the forest species that occurred (table 2). Although it is difficult to generalize because of site differences, *Abies religiosa*, *Pinus hartwegii*, *Pseudotsuga menziesii*, *Pinus ayacahuite*, *Quercus* spp., *Alnus* spp., and *Arbutus* sp. were among the tree species found at higher elevations (i.e., 2,500 to 3,500 m). At elevations below 2,500 m, *Liquidambar* spp., *Quercus* spp., *Platanus* spp., *Araucaria* spp., *Carpinus* sp., *Miconia* sp., and *Pinus* spp. were found. Many of the coniferous forests were managed using a system of partial cutting that removes a small proportion (about 40%) of the timber

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Cannon, Phil is a Regional Plant Pathologist at the USDA Forest Service Forest Health Protection, Region 5, 1323 Club Drive, Vallejo, CA 94593 USA. Klopfenstein, Ned B., Kim, Mee-Sook, and Hanna, John W. are Research Plant Pathologist, contract Plant Pathologist, and Biological Technician, respectively, at the USDA Forest Service – Rocky Mountain Research Station, Forestry Sciences Laboratory, 1221 South Main Street, Moscow, ID 83843 USA. Dionicio Alvarado Rosales is a Professor of Forest Pathology at Colegio de Postgraduados, Programa de Fitopatologia, Km. 36.5 Carr. México-Texcoco 56230. Montecillo, Edo. de México.

volume on a piece of ground about once every 12 years. We saw no evidence of clear-cutting.

Soils in these areas of Mexico are commonly derived from some sort of pyroclastic materials. In some locations, soils were composed of volcanic ash that had accumulated to great depths. On these deep ash sites, classic Andept soils were frequently found, especially at the cooler, higher elevations, where organic matter deposition outpaced organic matter mineralization. More commonly, however, soils found on this trip were composed of a shallow deposit of ash (10 cm to 1.5 m) on top of ignimbrite. Usually, this ignimbrite had been shattered into small rock fragments, so at least some portion of the trees root system could penetrate to significant depth. Ignimbrite (rock) was common near the soil surface on steep hogback ridges and these sites were sometimes associated with the occurrence of Douglas-fir. At similar elevations, *Pinus ayacahuite* and several species of oaks could be found on soils where deeper deposits of ash occurred, which was likely associated with more available soil moisture.

Although this trip was primarily focused on collecting *Armillaria* spp., other forest diseases were also observed. Some preliminary information and samples were also collected, which may provide useful information for establishing additional collaboration between Mexican and USA Forest Pathologists. It should be noted that *Armillaria* spp. had been reported previously on some trees in Mexico (see ahead); however, DNA-based identification of Mexican *Armillaria* spp. has not been previously reported.

Finally, the timing of this trip was also noteworthy. The trip began the week of September 9 after Hurricane Felix had passed through the area, and near the end of the rainy season. This circumstance was fortuitous for our collection work. The mushrooms and diverse fungal fructifications were quite abundant during our surveys. However, the local mycologists said that the peak season for collecting *Armillaria mellea* mushrooms begins 6 weeks earlier (i.e., early August); whereas *A. ostoyae* (or *A. polymyces*) mushrooms are more prevalent in December. Also, whole trees had been wind-thrown in many locations. This was particularly evident on the northern slopes of El Cofre de Perote, where wind gusts had reportedly reached 160 km/hr. These freshly wind-thrown trees, although a negative impact on the forest, provided a special advantage to our group because we could easily examine the base of the root wad and survey for fungi and rotten roots.

The Quest for *Armillaria* Species

Background

The research group that works for the Rocky Mountain Research Station in Moscow, Idaho (Ned, Mee-Sook and John), has been studying molecular diagnostics, genetic characterization, and evolutionary relationships among *Armillaria* species, especially *A. ostoyae* (Hanna and others 2007a, b; Kim and others 2000, 2001, 2006). These studies involve the DNA analyses of diverse *Armillaria* samples from several countries in the Northern Hemisphere. These studies, coupled with the fact that Mexico is well known as the center of diversity for many coniferous genera, which are the principal hosts of *A. ostoyae*, provided the impetus for the expedition to the coniferous forests of Mexico. The study of Mexican *Armillaria* isolates might provide insights to the southernmost extent of *A. ostoyae*, the origin of Northern Hemisphere *Armillaria* species, and evolutionary relationships among *Armillaria* spp. This baseline information is needed to evaluate the potential invasive species risk associated with Armillaria root disease pathogens. In addition, the epidemiology and ecology of the *Armillaria* pathogens in Mexico may provide insights into the future behavior of these pathogens in the USA as affected by climate change.

Methods

Some forest pathology research and related papers have reported the existence of *Armillaria* species in Mexico (e.g., Alvarado-Rosales and Blanchette 1994; Alvarado-Rosales and others. 2007; Murrill 1911; Pérez-Silva and others 2006; Shaw 1989; Tkacz and others. 1998; Valdés and others 2004). Other information on *Armillaria* spp. in Mexico is found in reports of edible mushrooms, commercial mushrooms, and ethnobotany (e.g., Montoya and others 2003; Montoya-Esquivel and others 2001; Ruán-Soto and others 2006). Specific information is also found in surveys of forest fungi and mycological forays, such as Ph.D. theses by Dionicio Alvarado-Rosales (in collaboration with Robert Blanchette) and Florencia Ramírez Guillen (in collaboration with Gastón Guzman). Currently, there are no reports of DNA-based diagnostics to identify *Armillaria* species from Mexico.

Four of the sample locations shown in table 2 were selected based on these earlier reports. Four other sample locations were selected because native Douglas-fir stands were known to exist in these areas, which represent the southern limits of this species. Douglas-fir stands were selected because of their relationship to forests of the western USA, and to examine whether *Armillaria* spp. and Douglas-fir had co-evolved. The remaining four locations were chosen because they were convenient and represented promising habitat for *Armillaria* spp.

At each collection site, *Armillaria* surveys were conducted by inspecting representative tree species present, regardless of health status. In addition, trees displaying potential symptoms of root rot were also inspected. Trees were inspected by excavating and pulling away all of the duff and soil from around the base of the tree and along two of the major roots to a depth of about 20 to 30 cm, using a small, sharp hoe. Brushes were used to clear away dirt that was in close contact with the roots. In cases where wood was infected, a hatchet or knife was also used to chop out small sections of the wood to reveal zone lines or wood rot in these sections.

The main *Armillaria* signs that were being sought were either mushroom caps or rhizomorphs (figure 2). When found, flexible rhizomorphs (ca. 10–60 cm) were collected from each tree, then placed into labeled, 15-ml collection tubes. Mycelial fans or decayed wood, with zone lines, were considered as an alternative source for *Armillaria* isolation, but it is more difficult to isolate this fungus from decayed wood. *Armillaria* mushrooms, mycelial fans, and/or rotted wood with zone lines were collected in labeled paper bags. All collected samples were placed in an ice chest until the fungus could be isolated. Once a tree was found with an acceptable *Armillaria* sign, associated collection data were recorded. Information gathered included a few photos of the tree, GPS readings (for latitude, longitude, and elevation), slope, aspect, host species, host symptoms, and associated plants. At the end of each collection effort in each of the three areas (Texcoco, Xalapa, and Oaxaca), one day was devoted to making isolations and establishing cultures.

The following procedure was followed when making the isolations from the rhizomorphs. The rhizomorphs were rinsed, in their collection tubes, with tap water to remove soil and other debris, the rhizomorphs were then soaked in a 20% Clorox® solution for 10 minutes, then rinsed with filter-sterilized, distilled water. Subsequently, they were soaked in a 3% hydrogen peroxide solution for 10 minutes and rinsed again with filter-sterilized, distilled water. Finally, the rhizomorphs were cut up into 1-cm-long sections and then placed into media slants within culture tubes. Two types of media were used: BDS (Benomyl-Dicloran-Streptomycin; Worrall and Harrington 1993) and "Very Cold *Armillaria* Medium" (VCAM; containing 1.5% agar, 0.75% malt extract, 0.5% peptone, and 0.75% dextrose).

Wood samples were treated by dipping the sample in alcohol and briefly flaming, then a small fragment was excised with a sterile scalpel. The fragment of infected wood was placed onto media slants within culture tubes. Similarly, with the mushrooms, small sections of fungal material were retrieved from the pileus or stipe that had first been alcohol flamed and then split open to allow the removal of small interior sections for culturing in the slants. Each isolate was cultured in 6 to 10 culture tubes containing BDS and VCAM.

Preliminary Results

Armillaria spp. were found in some sites at each of the three areas (Texcoco, Xalapa, and Oaxaca) that were visited; however, there were also some sites at each area where the fungus was not found. Where the fungus was found, it was usually found in very small infection foci (one to three trees); however, on one occasion we found a larger *Armillaria* infection center of approximately 1 hectare in extent. In general, we were more successful in finding *Armillaria* in the more humid areas, for example on alder, oak, or sycamore in valley areas as opposed to on the dry pine or Douglas-fir areas that occurred in high, dry slopes and ridges. Overall, 30 different sample collections were obtained; 23 of these were from rhizomorphs, 5 were from wood, and 2 were from fruiting bodies. It is especially noteworthy that *Armillaria* spp. were not found in association with Douglas-fir and were quite rare on true fir.

The Next Steps

At the end of this trip, all samples were sent to USDA-APHIS-PPQ where they were checked before they were delivered to the laboratory in Moscow. The shipment of *Armillaria* cultures from Mexico was in compliance with a USDA-APHIS-PPQ permit issued to the Moscow laboratory. In Moscow, each of the isolates that grows in culture will be sub-cultured onto Petri plates containing the same culture media. After a few weeks in culture, the isolates will be ready for DNA analyses.

In very general terms, DNA analysis involves using a small scrape of the mycelium from the culture plate, which serves as the DNA template for polymerase chain reaction (PCR). The PCR process allows one region of the rDNA (e.g., nuclear large subunit or intergenic spacer) to be amplified to produce millions of copies. The amplified region is then subjected to DNA sequencing, which allows comparisons among the isolates. The base sequence of the rDNA of these *Armillaria* collections from Mexico will be compared with the rDNA sequences of *Armillaria* from many other regions across the Northern Hemisphere. These comparisons of DNA sequences will allow determinations of the genetic relationships among many different genets and species of *Armillaria*.

Heterobasidion annosum

Heterobasidion annosum (previously *Fomes annosus*) has been previously reported in Mexico (e.g., Asiegbu and others 2005; Garbelotto and Chapela 2000; Guevara and Dirzo 1998; Johannesson and Stenlid J. 2003; Maloney and Rizzo 2002; Martínez-Barrera and Sánchez-Ramírez 1980; Ruiz-Rodriguez and Pinzon-Picaseno 1994; Sinclair 1964). Unfortunately, this pathogen has not been well characterized using DNA analysis. However, studies are currently underway to examine the phylogenetic relationships of *Heterobasion annonum* P-type intersterility group (P ISG) from Mexico with other members of this group from North America and Eurasia (Linzer and others 2007).

On this trip, *Heterobasidion annosum* was first found on a *Pinus patula* stump (figure 3). The location was the Ciclo Verde Christmas Tree Plantation near Xalapa. The stump was 40 cm in diameter and was located on the top of a ridge. A brief search revealed a few more *P. patula* stumps with *H. annosum* conks. The conks were typically quite small with about 1–2 cm^2 worth of freshly sporulating surface per conk (figure 3). Many of the native *P. patula* in this area had recently been felled in an intensive selective cut that left about 40 well-spaced trees per hectare. The wood from the felled trees was processed for lumber and Christmas trees were planted in the understory of the residual trees (the species planted for this purpose include *Cupressus lusitanica, Pinus ayacahuite*, and *Pseudotsuga menziesii*). A few of the residual trees had blown over as a result of Hurricane Felix, which had reportedly delivered winds of 160 km/hr through this stand. This afforded an easy opportunity to study the base of the roots in these upturned trees. In one of these situations, we found that several of the principal “tap” roots had been severely decayed, and *H. annosum* fructifications were observed on the stump and on the root system. The Annosus root disease had pre-disposed this tree to wind-throw. Further indications were that this root disease was becoming a new problem in this area. No well-established, root-rot foci were found, and none of the stumps examined showed much more than incipient decay.

Although this disease is not well known in Mexico, it seems likely that the management practices at Ciclo Verde could have contributed to the recent build-up of the pathogen.

More importantly, the build up of Annosus root disease could have important long-term consequences on the native *P. patula*. This pathogen also represents a special problem for the *P. ayacahuite* in the Christmas tree plantation, as it can spread by root contact or by spore-derived infections of freshly cut stumps. We talked with the foreman, Don Lupe, about the problem. He listened carefully, and he was eager to learn more about possible control measures.

Unknown Root Disease of True Fir

An unknown root-rot pathogen was found infecting *Abies religiosa* growing at high-elevations in Tlaxcala and Hidalgo. This root disease was apparent in wind-thrown and dead/declining trees. No fruiting bodies were observed; however, white mycelia were apparent under the root bark of infected trees (figure 4). Further investigation of this disease is warranted, and perhaps rDNA sequencing can help identify the unknown pathogen.

Rust

Forest pathologists in the USA (e.g., Brian Geils, USDA Forest Service) are establishing collaborations with forest pathologists in Mexico to assess the risk of white pine blister rust (*Cronartium ribicola*) to any of the five-needled, white pines that are native in Mexico. To date, white pine blister rust has not been reported for Mexico. However, blister rust does occur on *Pinus strobiformis* in New Mexico (Hawksworth 1990; Van Arsdel and others 1998; Conklin 2004), and *P. strobiformis* also occurs in the northern half of Mexico. Several other species of five-needled, white pines occur at diverse locations in Mexico.

To assist in the effort to assess risks of white pine blister rust, we briefly inspected *Ribes* and *Castilleja* plants. *Ribes* plants were found in essentially every area that we visited, except Oaxaca, and *Castilleja* spp. were common in most areas (figure 5). We commonly found uredinial pustules on *Ribes* leaves at several locations. The GPS location of these occurrences was recorded and photos were taken of the rust infections and *Ribes* plants. The identification of this rust remains unknown; however, it appeared widespread within the areas that we visited. Preliminary observations indicated that the rust could potentially belong to the *Coleosporium* genus; however, DNA-based diagnostics are needed to conclusively identify the rust pathogen of *Ribes* spp. The identification of rusts in Mexico may help assess risks for white pine blister rust. Also, we never observed any symptoms of rust on any of the white pines; however, time did not permit more that a cursory inspection of the white pines. In regard to risk of white pine blister rust in Mexico, it must be noted that the use of susceptible *P. ayacahuite* is quite common in Christmas tree plantations at diverse locations in Mexico (figure 6). These plantations may significantly influence the risks associated with white pine blister rust. Furthermore, it must also be considered that rust could potentially “over-winter” as uredinia on *Ribes* in Mexico, thereby maintaining a pathogen population in the absence of aecial host infection.

Oak Wilt

Surveys are needed to determine whether oak wilt occurs in Mexico (Appel 1995). Although we saw thousands of Mexican oak trees of diverse species during this visit, we did not see any with symptoms of oak wilt. Indeed the vast majority of oaks that we looked at seemed quite healthy (figure 7). However, it cannot be ruled out that oak wilt could occur in Mexico, and it is unknown if oak wilt-infected trees will display typical oak wilt symptoms in Mexico.

Xylella fastidiosa

Xylella fastidiosa is a bacterial pathogen that causes scorch and dieback in a number of forest trees, fruit trees, vines and ornamental plants in the USA (Hopkins 1989). In forestry, it is well known for its effects on sycamore (*Platanus* sp.) and sweetgum (*Liquidambar* sp.). On sycamore, it causes a devastating disease on trees that are 4 years old and older throughout most of the southeastern USA. Breeding programs to develop resistance to this pathogen are underway (in the case of sycamore) and are being considered (in the case of sweetgum). Mexico is known as a source of potentially resistant germplasm for both of these species and their nearby relatives.

During this trip, several hundred sycamore and sweetgum trees were observed (figure 8). None of these showed apparent symptoms that are commonly associated with *Xylella fastidiosa*. It is unknown whether the absence of leaf scorch was because hosts are resistant to the disease/insect vector or whether the pathogen or its most common vectors (glassy-winged sharpshooters) are not present in this environment. However, we did not see any evidence to indicate that these Mexican sycamore and sweetgum sources were susceptible to this pathogen.

Dwarf Mistletoe

Dwarf mistletoe was extremely abundant in most of the conifer stands that we visited and was perhaps most abundant in forests that were being managed for timber production via a system of partial cutting (figure 9). Some of these forests were even certified by the Forestry Stewardship Council (FSC) as being model forests. The very heavy brooming that is commonly found when dwarf mistletoes are established on some conifers was sometimes observed, indicating that these mistletoes are exerting a burden on their coniferous hosts. The practice of partial cutting, which allows infected trees to rain vast numbers of dwarf mistletoe seed down on the regeneration that follows the harvest, may be contributing to heavier levels of infection.

Dwarf mistletoe has been recognized as a serious problem in many coniferous forests of the USA for many decades and control measures have been designed to contend with this problem (Hawksworth and Wiens 1996; Geils and others 2002). It is likely that many of the management measures that are used in the USA would also be useful in Mexico. The translation of several English-language documents on this subject into Spanish is perhaps one of the simplest measures to help make some of the effective management measures more available in Mexico. It is worth noting that Frank Hawksworth, the world's leading authority on dwarf mistletoes, made several trips to Mexico to document the species of this pathogen in the coniferous forests. Currently, Bob Mathiasen (Northern Arizona University, taxonomy and distribution) and Brian Geils (USDA Forest Service-RMRS, epidemiology) have expertise to work with dwarf mistletoes in Mexico. Forest Health Protection pathologists from Regions 2 and 3 are working with cooperators in northern Mexico on a dwarf mistletoe–silviculture project (see Howell and others, this proceedings).

Bark Beetles

Little evidence of bark beetles was observed during this trip; however, we did see some bark beetle-attacked trees 10 km northeast of San Miguel del Valle, and they were, as might be expected, on the drier parts of the landscape. According to Demitrio Policarpo Santiago (Oaxaca), their company removes bark beetle-impacted trees during timber harvest (figure 10). This management practice appeared to be efficacious, although it requires extra effort during the harvest period to access these dying trees that are typically scattered across the landscape. Previously, we mentioned that partial cuts are likely a contributing factor to the dwarf mistletoe problem. To the contrary, in the case of bark-beetles, partial cutting and rapid removal of bark beetle-infested trees should relieve between-tree competition that can exacerbate further infestations of bark beetles.

Previous and ongoing collaborations among Mexican and USA forest entomologists may have helped to develop sound management practices to reduce bark-beetle problems. Regardless of how these practices came about, it was apparent the coniferous forests where we visited have been well managed from a stand density point of view. This management likely contributed significantly to the overall health of all the residual trees in the forest.

Climate Change

Because our trip occurred toward the end of the rainy season, the areas we visited were at their absolute greenest. During our travels, we rarely observed forested areas that were experiencing higher than normal levels of natural mortality. This was somewhat surprising since mortality rates in coniferous forests of some other areas has became exceedingly high within the last few years (e.g., about 50% mortality of mature coniferous trees has been observed in National Forests in southern California during the past 5 years).

The local foresters in Mexico indicated that the past 5 years, including the current year, have been far drier than previous years. One forester commented, "The rains are disappearing in September now, whereas they used to go until the end of October." Still, the drought situation in sites that we visited has apparently not resulted in the severe impacts that have been recently observed in the southwestern USA.

The Douglas-fir stands in Oaxaca represent the southernmost extent of this species (Delbreczy and Rácz 1995). These “island” populations are at the limit of the species range, and thus may be most at risk to climate change. Some mortality was found in these stands, but root rot was not found (figure 11). The mortality was associated with several years of dry weather, which may be associated with changing climate. The most common symptom observed in these Douglas-fir stands, however, was a paucity of older foliage and smaller duskier tufts of needles at the branch ends. This, too, is very symptomatic of extreme and sustained drought, but the trees so far have demonstrated remarkable resilience.

Future Collaboration

Many strong opportunities exist for collaboration among Mexican and USA forest pathologists. Although many Mexican forest pathologists and related professionals can speak English, Mexico–USA collaborations would benefit substantially from bilingual (English–Spanish speaking) forest pathologists in the USA. Because of common interests, the establishment of bilingual forest pathology positions should be encouraged in the USA to maximize information exchange with Mexico and Latin America.

The DNA-based studies of *Armillaria* may result in collaborative publications and may raise new phylogenetic questions that warrant further study. Forest pathologists from both countries need to understand the distribution and ecological behavior of diverse *Armillaria* species and subspecies. This baseline information will help understand risks of hybridization and/or invasiveness, and shed light on potential interactions with climate.

The incipient *Heterobasidion annosum* situation would benefit greatly from information sharing and collaboration. Collaboration among USA and Mexican forest pathologists and foresters should promote information exchange about measures that can be successfully deployed to limit the spread of this disease, while it is still in the incipient stage. The USA has extensive experience in learning how to manage this disease in the southeastern and western forests. However, forest pathologists in the USA need to understand the ecological behavior of *H. annosum* in Mexico. Matteo Garbelotto (University of California, Berkeley) is currently studying the phylogenetic relationships for *H. annosum* from Mexico in comparison with those found in the USA and other Northern Hemisphere regions. This work is important to understand the identity and ecological behavior of these pathogens.

Similarly, the USA and Mexico could benefit by collaboration and information sharing toward reducing the impact of dwarf mistletoe diseases (e.g., eight USA states have fairly aggressive dwarf mistletoe programs).

Rust was evident on *Ribes* in many locations, but we did not see any evidence of rust on coniferous hosts. Further investigation may be needed along these lines involving rust pathologists with the necessary field skills for monitoring this disease. The identification of the rust on *Ribes* could help assess the risk of white pine blister rust, and help determine whether suitable environment for white pine blister rust occurs in Mexico.

Extensive other opportunities exist for forest pathology collaboration between Mexico and the USA, but these opportunities lie outside the initial focus of our preliminary observation during our *Armillaria* collection trip (figure 12).

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Table 1—Mexican and USA collaborative contacts for *Armillaria* collection trip.

Principal Contacts Name	Expertise	Affiliation
Dionicio Alvarado Rosales	Forest Pathology	Colegio de Postgraduados, Texcoco
Carlos Martinez	Plant Pathology	Colegio de Postgraduados, Texcoco
Rosario Medel	Mycology	Instituto de Ecologia, Xalapa
Patricia Negeros-Castilla	Forestry	Universidad de Xalapa, Xalapa
Florencia Ramírez Guillén	Mycology	Instituto de Ecología, Xalapa
Gastón Guzman	Mycology	Instituto de Ecología, Xalapa
Armando López	Mycology	Instituto de Genética Forestal
Elfego Chávez Gonzáles	Forestry	Consultor Privado, Oaxaca
Marcario Pérez López	Forestry	Consultor Privado, Oaxaca
Victor Hernández Guzmán	Forestry	Gerente de la Empresa Forestal La Cumbre
Félix Luis Ramírez	Forestry	Mayordomo El Carrizal
Alejandro Hernández	Forestry	Vice President San Miguel Del Valle
Demitrio Policarpo Santiago	Forestry	Treasurer, San Miguel Del Valle
Additional Contacts		
Efren Cázares	Mycology	Oregon State University (via Monterrey)
Jesús García Jiménez	Mycology	Universidad de Tamaulipas
Gonzalo Guevarro	Mycology	Universidad de Tamaulipas
Miguel Angel Muniz Castro	Mycology	Instituto de Ecología, Caseres
Matt Smith	Mycology	Harvard University (from Dave Rizzo lab)
Greg Bonito	Mycology	Duke University (Rytas Vigalys' lab)
Jim Trappe	Mycology	Consultant (via USDA FS, Corvallis, OR)

Table 2—Field locations visited during this trip

Locations in Mexico	Examples of host genera sampled	Elevation (m)	Accompanied by
Near Texcoco, Mexico State			
Popocatépetl	*Abies, Ribes*	2,900 – 3,300	Dionicio Alvarado Rosales
Parque Nacional El Chico	*Abies, Pinus, Pseudotsuga*	2,800	Dionicio Alvarado Rosales
Villarreal	*Abies, Pinus, Ribes*	3,300	Carlos Martinez
Bosque University of Chapingo	*Abies, Alnus, Pinus, Senecio*	3,400	Dionicio Alvarado Rosales
Near Xalapa, Veracruz			
Mesophyll Caceres	*Carpinus, Liquidambar, Miconia, Quercus*	1,500 – 1,600	Rosario Medel
Cofre de Perote	*Abies, Pinus, Ribes*	3,500 – 3,600	Rosario Medel
Reserva Ecológica, San Juan del Monte, south of Las Vigas	*Alder, Pinus*	2,300	
Instituto Genética Forestal, El Haya Parque Ecológíco, Xalapa	*Platanus, Quercus*	1,400	Armando López
Asseradero 100, Xalapa	*Araucaria*	1,400	Armando López
El Ciclo Verde Christmas tree farm, Las Vigas	*Pinus*	2,500	Rosario Medel
Areas near the “Hayas parking zone”, Instituto de Ecologia, A.C.	*Eriobotrya, Platanus, Quercus*	1,300	Rosario Medel
Near Oaxaca, Oaxaxa			
Peña Prieta, in Parque La Cumbre - Ixtepeji	*Arbutus, Pinus, Pseudotsuga, Quercus*	2,800	Marcario Pérez López
El Carrizal, 10 km north-east of San Miguel Del Valle	*Alnus, Pinus, Pseudotsuga, Quercus*	2,600 – 2,800	Demitrio Policarpo Santiago

Figure 1—Locations visited for *Armillaria* collection trip in Mexico

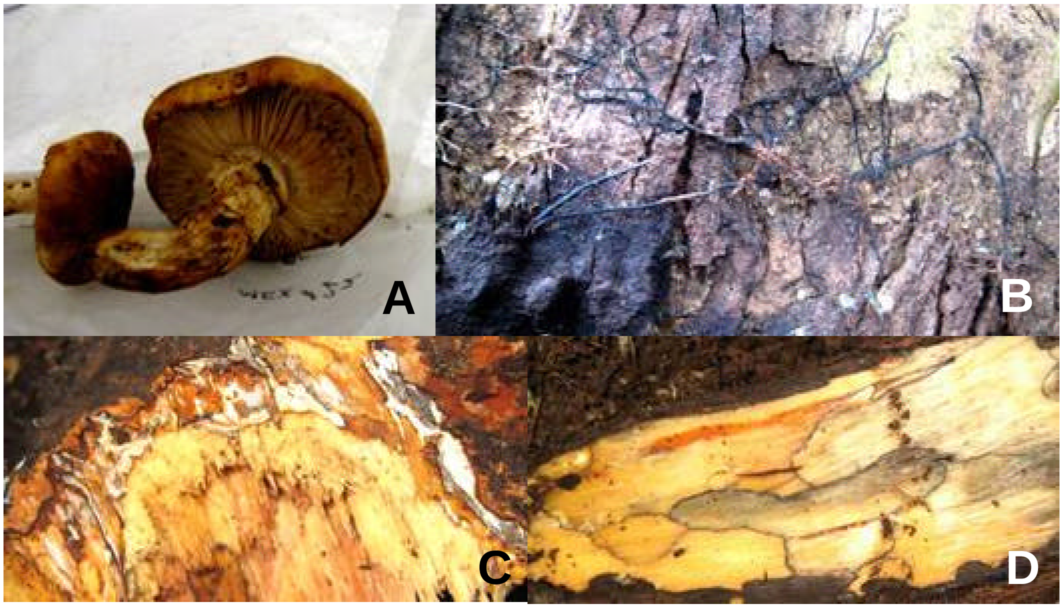

Figure 2—Signs of *Armillaria* spp.: fruiting body (A); rhizomorphs (B); mycelial fans (C); and zone lines (D).

Figure 3—*Pinus patula* stump in the Ciclo Verde Christmas Tree Plantation, Xalapa, Mexico (A); fruiting body of *Heterobasidion annosum* found on stump (B).

Figure 4—Unknown fungal pathogen on *Abies religiosa* roots

Figure 5—Infected *Ribes* leave with rust uredinial pustules (A) and *Castilleja* plants (B)

Figure 6—Native *Pinus ayacahuite,* which is potentially susceptible to white pine blister rust. A native tree near Oaxaca, Mexico (A), and a Christmas tree plantation near Xalapa, Mexico (B).

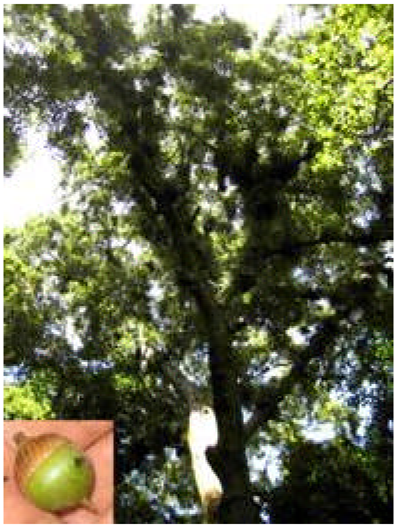

Figure 7—One example of many *Quercus* species found in Mexico.

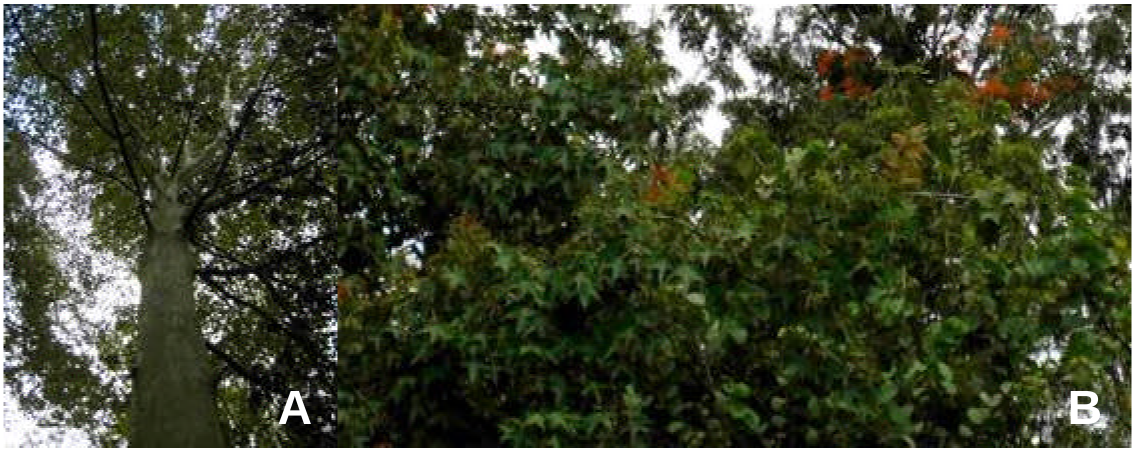

Figure 8—Healthy sycamore and sweetgum

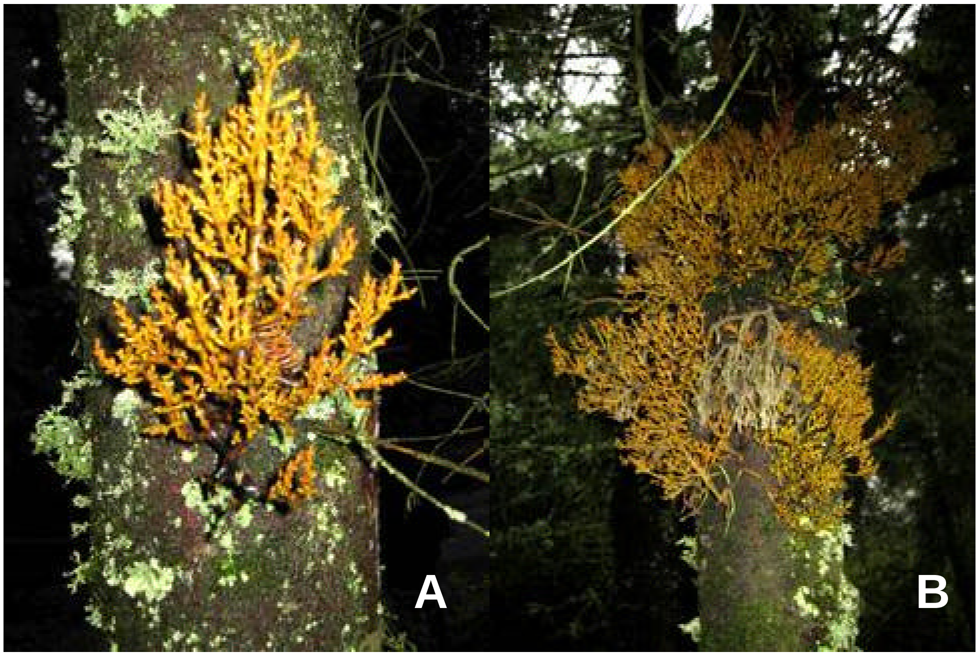

Figure 9—Dwarf mistletoes

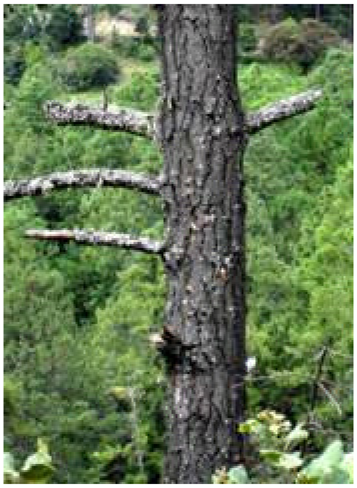

Figure 10—An example of bark beetle damage to *Pinus* sp. in Oaxaca

Figure 11—Douglas -fir stands in Oaxaca, Mexico. Some mortality was found in these stands perhaps due to climate change (prolonged drought) (A). Pine mortality also potentially associate with climate change (B)

Figure 12—Interactions with our Mexican contacts at various collection sites and local institution in Mexico.